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RELATIONS BETWEEN METABOLISM AND MORPHOGENESIS DURING REGENERATION IN TUBIFEX TUBIFEX I

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There have been two prime viewpoints regarding the relation of metabolism to morphogenesis. One advanced by Child and his students has considered rate of metabolism in relation to the organismal factors initiating and directing the course of morphogenesis. Barth (1938, 1940a, 1940b), supporting Child's view, has suggested that increased oxygen tension in the tissues adjacent to a wound may furnish a primary stimulus for regeneration in hydroids; but he found that rate of regeneration was much more markedly stimulated by high oxygen tension than was rate of oxygen consumption. Thus the effect on regeneration may not have been mediated through an effect on total aerobic metabolism. The other viewpoint, advanced by Tangl (1909) and more recently by Tyler (1933, 1936, 1942), has considered rate of metabolism in relation to the release and utilization of energy in morphogenetic processes, particularly differentiation. Tyler concluded from his work on sea urchin embryos that differentiation requires the expenditure of metabolically released energy in addition to that required for maintenance, and that this energy does not become resident in structure but is released as heat during morphogenesis. This view is consistent with the work on "activity metabolism" (cf., Fisher et al., 1942, 1944).

The present study was undertaken to analyze further the relations between metabolism and morphogenesis from both viewpoints. In the regenerating annelid, measurements of morphogenesis may be made by counting the number of new segments produced (Stone, 1932 and Coldwater, 1933). The formation of a new segment is not a simple event, but involves distinct stages. This allows estimation of the rates at which various processes in regeneration proceed. Metabolism may be separated into fractions by means of poisons, and the relation of the activity of particular cellular respiratory systems to morphogenesis may then be tested. Two modes of approach were used in this study: oxygen consumption and respiratory sensitivity to poisons were measured at various times during regeneration (results reported in this paper), and the effects of continuous poisoning and of high and low oxygen tension were determined by measuring the progress of regeneration (results to be reported later).

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Materials and Methods

Worms of the species *Tubifex tubifex*[1] were rigorously selected for uniform size and condition (3.5 to 5.0 centimeters in length, a light, smoothly graded color, no signs of previous regeneration, no signs of breeding condition). For a week before use and between determinations the worms were kept in frequently changed tapwater at about 17°C. A 0.2 per cent solution of chloretone was used as an anaesthetic during amputation of the posterior two-fifths of each worm and during examination under the microscope. Individual worms were kept in anaesthesia no longer than ten minutes every third day (cf., Stone, 1932). Stage and rate of regeneration were estimated at intervals of several days. Three stages in the formation of a new segment were clearly distinguishable *in vivo*. First the new segment appeared as a narrow shadow perpendicular to the longitudinal axis of the worm. Later the new segment was slightly longer and the septum appeared as a pair of fine sharp lines. Finally the new setae were visible as small refractile bodies. The three stages were considered to be stages of localization, early differentiation, and later differentiation respectively (cf., Stone's description of the cytology and histology of regeneration in *Tubifex tubifex*). The number of segments in each of the stages was recorded separately. After regeneration had proceeded for a week or more, all stages of segments were present in the new tail, but segments which were hard to classify were few in number. "Rate of localization"[2] was calculated as the increase in total number of segments per worm per day; "rate of early differentiation" as the increase in number of segments in all stages of differentiation, and "rate of later differentiation" as the increase in number of segments with setae.

Most of the determinations of oxygen consumption were made by the Warburg manometric method, at 25°C. Worms for these determinations were taken in groups of twenty-five to forty, collected into a ball, rolled on filter paper to free them of excess water, placed in a pan of platinum foil and weighed quickly on a torsion balance. Consecutive weighings were found to deviate by less than one per cent. A difficulty in use of intact animals in the Warburg apparatus was encountered in the differences in degree of dispersion of the worms over the bottom of each flask. Probably the marked ability of *T. tubifex* to contract "oxygen debt" (Harnisch, 1935) was brought into play when the worms remained in a ball (average oxygen consumption in five determinations during which the worms remained in a ball was 0.12 milliliter per gram wet weight per hour compared to 0.16 milliliter for dispersed worms). Fortunately the worms spread out uniformly in most cases and only those determinations throughout which the worms were uniformly dispersed were included when calculating averages.

Successive determinations were made on the same worms at intervals of several days and the progress of regeneration was also measured. That this routine was not unduly injurious to the worms was indicated by a normal rate of regeneration and a casualty rate only slightly higher than that of worms upon which no manometric determinations were made; also the oxygen consumption of intact worms used as control remained constant within the limits of error throughout each series of

[1] Identification confirmed by Dr. R. G. Stone, University of Kansas City.

[2] This is the same as rate of regeneration as used by Stone (1932) and Coldwater (1933), although these authors did not calculate rate on a daily basis. From their published data on comparable worms the curve in Graph 1 may be duplicated.

determinations. These intact worms were anaesthetized and examined as were the experimental animals, injured individuals being removed from the group. About 10 per cent of the worms in each group had been removed by the end of the experiments.

A modification of the method reported by Howland and Bernstein (1931) was used for measuring oxygen consumption by individual worms. Each worm was drawn into a capillary tube 0.5 millimeters in diameter. By shifting the worm in a drop of water back and forth in the tube, air was drawn in at each end, then a drop of 5 per cent potassium hydroxide solution. Finally one end was sealed with paraffin oil and the other with vaseline (see Fig. 1). The tube was mounted in a

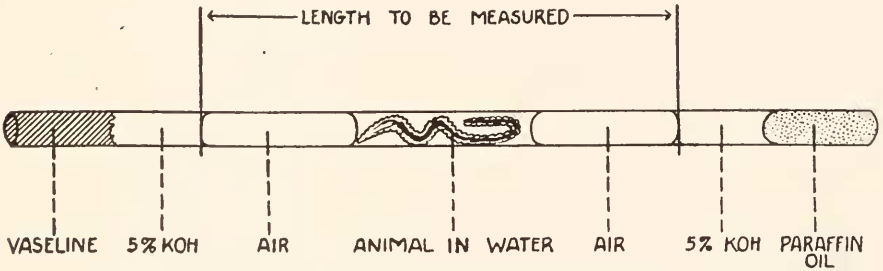


FIGURE. 1. Diagram of respirometer used in modified Howland-Bernstein method of measuring oxygen consumption (much enlarged).

vertical position on a glass plate in a glass-walled constant-temperature bath at 21°C. About fifteen such tubes were handled at a time. The distance between upper and lower air-KOH interfaces was measured using a horizontal microscope with a calibrated adjustment. The inside diameter of the capillary was measured exactly and the volume of oxygen consumed was calculated directly. A wormless tube served as a thermobarometer. The movement of the worm in the tube was found to furnish an efficient stirring mechanism in the fluid part of the system, and it was calculated that the capillary was not fine enough to hinder diffusion in the gaseous phase. After a series of readings, the capillary tubes were broken at the air spaces and the worms removed uninjured. Measurement of each worm was then carried out by anaesthetizing it and taking the diameter at four or five points and the length. The volume of the worm was computed as a series of cylinders.

DATA ON WORMS NOT UNDERGOING REGENERATION

As determined both by use of the Warburg apparatus and by the method just described, the rate of oxygen consumption of normal worms was within the range of rates reported by other workers using various methods (Dausend, 1931; Harnisch, 1935; Brazda and Rice, 1940). In agreement with the last named investigators, it was found that size of worms from 2.5 to 5.0 centimeters in length made no significant difference in rate of oxygen consumption. Concentrations of potassium cyanide from 2×10^{-3} M to 2×10^{-6} M had no inhibitory effect on oxygen consumption by normal worms (see Table I). Cyanide in a concentration of 2×10^{-2} M was rapidly lethal, but nevertheless did not decrease oxygen consump-

tion as drastically as its toxicity might suggest. Comparison with the data reviewed by Commoner (1940) shows that the total oxygen consumption by *T. tubifex* is of the same absolute magnitude as the cyanide-stable fraction of respiration in many other animals and tissues for which such data are available.

TABLE I

Oxygen consumption by intact worms in various concentrations of potassium cyanide

Solution	Q_{O_2}	Number of determinations
Tapwater	0.16	22
2×10^{-6} M KCN	0.16	2
2×10^{-5} M KCN	0.16	6
2×10^{-4} M KCN (non-lethal)	0.15	5
2×10^{-3} M KCN (semi-lethal)	0.19	2
2×10^{-2} M KCN (lethal)	0.11	1

Note: Determinations were made using standard manometric procedure at 25°C.

Respiratory quotient was found to average 0.68 for normal starving worms. The data agree with those of Brazda and Rice and suggest non-carbohydrate metabolism, possibly protein or fat metabolism. Such a type of metabolism is also expected from the cyanide-stable character of the respiration (cf., Commoner's review) as well as from the starving condition of the worms.

EXPERIMENTAL RESULTS

In preliminary work measuring the oxygen consumption of individual worms (see Table II) it was found that during the first few days of regeneration oxygen

TABLE II

Oxygen consumption by individual worms

Condition	Oxygen in mm.³ per mm.³ of worm per hour					
	0 days	1 day	8 days	11 days	15 days	19 days
Normal intact	0.10	0.10	0.11	0.09	0.09	0.09
Normal cut (regenerating)	0.10	0.12	0.14	0.17	0.15	0.15
X-rayed intact	0.08	0.09	0.07	0.09	0.08	0.11
X-rayed cut (inhibited)	0.10	0.11	0.09	0.11	0.10	0.11

Note: Determinations were made using capillary tubes at 21°C. Probable error was ±0.01.

consumption proceeds at a rate only slightly higher than normal. Later a marked increase in rate of oxygen consumption appeared: 0.08 mm.³ per mm.³ of worm per hour, or 85 per cent above normal. Subsequently the rate of oxygen consumption decreased. It was found that worms in which regeneration had been inhibited

by X-ray treatment showed no similar increase in oxygen consumption. It was considered that the increased oxygen consumption was associated with some process occurring during regeneration. In this preliminary work no counts of segments had been made, but the data of Stone (1932) were used for comparison. It was suggested that differentiation might be the process with which the increased oxygen consumption was associated. It was thought possible that the increase in oxygen consumption might be cyanide-sensitive as in the case of grasshopper embryos (Bodine et al., 1934, 1940). To test this possibility as well as to check results thoroughly using a standard method, experiments were set up in which the Q_{O_2} of regenerating worms in tapwater and in dilute cyanide solution (2×10^{-4} M KCN) was determined using Warburg manometers. Detailed observations of the progress of regeneration were made on the same worms. A total of nine groups of worms, 303 individuals, were followed through regeneration in three series of experiments. Four groups of worms, 137 individuals, served as control. Results of the manometric determinations are summarized in Table III, each figure being the average of

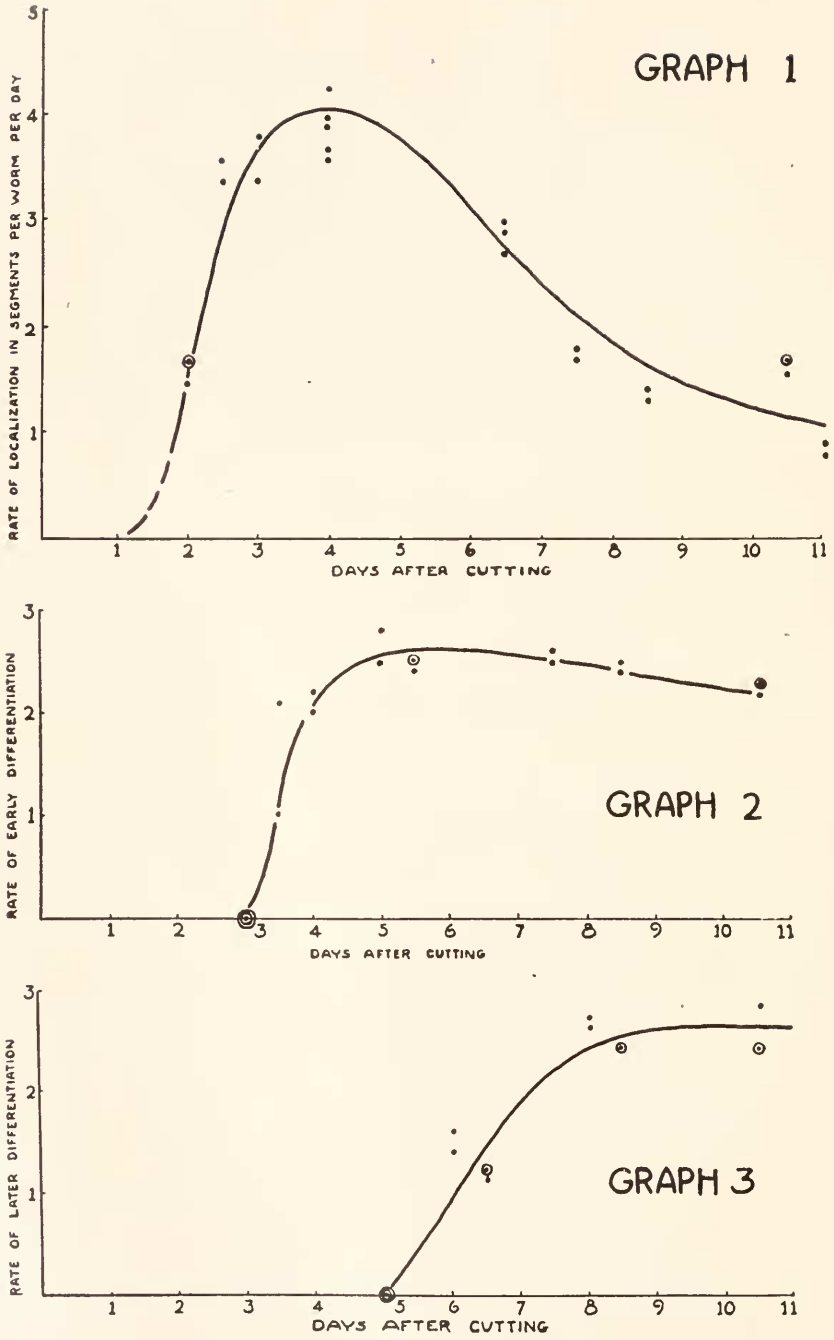
TABLE III

Oxygen consumption by groups of worms

Days	Predominating regenerative processes	Regenerating worms		Concurrent control	
		Q_{O_2}	$Q_{O_2}^{KCN}$	Q_{O_2}	$Q_{O_2}^{KCN}$
2	Mobilization of neoblasts and beginning localization	0.18	0.20	0.17	0.17
3	Localization	0.17	0.16	0.16	0.16
4	Localization	0.16	0.15	0.16	0.15
5	Localization and early differentiation	0.17	0.16	0.16	0.15
6	Early differentiation	0.16	0.15	0.17	0.17
8	Early differentiation	0.18	0.17	0.16	0.15
12	Later differentiation	0.24	0.24	0.16	0.15
13	Later differentiation	0.24	0.25	0.15	0.15

Note: The error when calculating from data on thirty-minute intervals was ±0.009. When calculating from data on the whole run, the error was reduced to about ±0.003. Mean deviation of separate averages from the grand averages listed in this table was ±0.008.

determinations on from two to five groups of worms. Data on progress of regeneration are summarized in Graphs 1, 2, and 3. After two days of regeneration, during which localization occurred in the first new segment from the neoblasts in the blastema, oxygen consumption was slightly above normal. Between three and five days, the period of maximal rate of localization, oxygen consumption was only slightly if at all above normal. While early differentiation was proceeding at its maximum rate six days after removal of the tail, oxygen consumption was strictly normal. But while later differentiation was proceeding, rate of oxygen consumption was increased: at twelve days the rate was 0.08 milliliters per gram per hour higher than the control, i.e., 50 per cent higher. At this time rate of later differentiation was at a maximum. The determinations using the Warburg method confirm in



Note: Mean deviation within each group of worms (twenty-five to forty individuals) ranged from 0.2 to 0.4 segments per worm per day, increasing with time.

general the results of the earlier determinations using individual worms in capillary tubes.[3] At no time was a significant cyanide-sensitive fraction of respiration found.

A summary of data on loss of weight by the worms involved in this study is given in Table IV. These data indicate not only that the regenerating worms lost

TABLE IV

Original length of worms in centimeters	Condition	Average weight per worm at first weighing after removal of tail (2, 3, 4 days)	Average weight per worm at last weighing (12, 13 days)	Average percentage loss per day
3.5 to 4.0	Regenerating	1.36 mg.	0.94 mg.	3.09
	Normal	1.92 mg.	1.57 mg.	1.82
4.0 to 5.0	Regenerating	2.07 mg.	1.57 mg.	3.01
	Normal	2.53 mg.	2.22 mg.	1.51

almost twice as much weight as the control, but also that the initial differences in weight between experimental and control groups due to removal of the tails cannot account for either the increased oxygen consumption or the increased loss of weight. The latter point is clear from a comparison between the larger regenerating worms (4.0 to 5.0 centimeters original length) and the smaller normal worms (3.5 to 4.0 centimeters in length).

DISCUSSION

Morphogenesis, the origin of form, involves the production of the specific structural chemicals of protoplasm, their orientation into submicroscopic structure, and the summation of submicroscopic structural differences into microscopic and macroscopic structure. Morphogenesis depends upon metabolism in that anabolic processes produce the particular compounds which become oriented into structural parts and catabolic processes must release in usable form such energy as may be required, if any, in morphogenetic processes. Morphogenesis may involve work in the thermodynamic sense according to either of two conditions: (1) there may be free energy of formation of the structure such that the energy becomes resident in chemical and physical structure, and/or (2) the synthesis of materials, their transport to particular positions, and their fixation in these positions, may require energy in such a way that the energy is ultimately dissipated as heat, even as the energy required to move an object horizontally from one point to another is ultimately dissipated as heat.

Tangl (1909), Farkas (1903) and Bohr and Hasselbalch (1903) defined "Entwichlungsarbeit," "work of development," as the total energy dissipated during embryonic development. In a number of different animals the Entwicklungsarbeit was found to be equal to about one-third of the total energy content of the unincubated egg. Needham (1931) criticized the concepts of Tangl and contended that

[3] The differences in general level of oxygen consumption are probably due to the difference in temperature at which determinations were made, i.e., Warburg determinations at 25° C., and capillary tube determinations at 21° C. (Brazda and Rice, 1940, found that oxygen consumption was increased about 40 per cent when the temperature was raised from 25° C. to 30° C.) and to inequality of unit volume to unit weight.

"work of development" should be a potential energy resident in structure, i.e., should be "work of differentiation." He further pointed out in reviewing the work of Bohr and Hasselbalch, that the energy resident in mechanical structure must be either non-existent or extremely small in amount. Essentially, the difficulties arose from the inadequacy of controls: no measure of an energetic cost of maintenance separate from an energetic cost of development was available in the work of Tangl, Farkas, and Bohr and Hasselbalch.

Tyler (1933) found that normally developing dwarf sea urchin embryos consumed more oxygen than normal-sized embryos in reaching the same stages of development. He interpreted his results in terms of a metabolic cost of differentiation. Tyler's concept, different from that discussed by Needham, involves the expenditure of energy in morphogenesis but not necessarily the storage of energy in visible structure. His investigations of giant embryos gave evidence consistent with his interpretation and he tried further to dissociate differentiation, growth, and maintenance in his material, but was unsuccessful. He stated (1936), "It has been concluded from earlier work that energy is required for the processes of embryonic differentiation, although the quantities involved could not be estimated."

Some of the difficulties encountered in studies of morphogenesis in embryonic development are avoided when morphogenesis is investigated in regeneration. Intact animals may be used in measuring the metabolism associated with maintenance,[4] and comparison between the metabolic rates of regenerating and of intact animals gives a measure of metabolism associated with morphogenesis. Increased rate of oxygen consumption during regeneration has been reported in Planarians (Coldwater, 1930). The additional metabolism appeared immediately after cutting and may have been associated with proliferation and increased numbers of the formative cells in this case. Coldwater chose this interpretation. However, it has not been demonstrated that either formative cells or other cells of an embryonic nature have intrinsically high respiratory rate. Tumor cells, for instance, have been found to have a relatively low Q_{O_2} (Warburg, 1930). Since these may be considered embryonic cells in which determination and differentiation are not proceeding, their metabolism may be considered entirely associated with maintenance and proliferation. On this basis, maintenance of embryonic cells should not, in itself, lead to increased respiration. From an examination of Needham's comprehensive review of the investigations of metabolism of embryos it is clear that the magnitude of rate of oxygen consumption is not greater than that of certain adult tissues. It is possible that in regenerating Planarians the additional metabolism was associated with truly morphogenetic processes rather than with proliferation and maintenance, but the data do not allow a distinction between the possibilities.

In the present work a distinction is possible. During regeneration in *T. tubifex* the rate of oxygen consumption was found to remain normal for the first week. During the second week the oxygen consumption was found to increase to well above normal. Because the markedly increased oxygen consumption did not appear during early stages of regeneration, it certainly was not associated with mobilization or proliferation of the neoblasts. That it was not associated with increase in size

[4] In the present work the data provide an empirical check on this assumption: since the Q_{O_2} remained normal during most of the first week of regeneration, the aerobic metabolic cost of maintenance of regenerating worms must be the same as that of intact worms.

of the new segments is clear from the consideration that increase in rate of oxygen consumption appeared before marked increase in the size of the new tail; during the first two weeks the regenerant tail constituted only a very small fraction of the total volume of the worm (less than one-twentieth). Hence the additional oxygen consumption may be considered to be associated with morphogenesis proper. From the present data it is not possible to distinguish whether the additional oxygen consumption occurred in the regenerant tail alone or whether the older tissues also consumed oxygen more rapidly. If the increase be referred to the regenerant tail alone, the Q_{O_2} of this part must have been extremely high. However, in estimating a metabolic cost of morphogenesis it is logical to refer the cost to the worm as a whole. Estimated on the basis of oxygen consumption, the metabolic cost of morphogenesis during the second week of regeneration would be at least half as great as the cost of maintenance of the whole worm. Another estimate of a possible cost of morphogenesis is available from the data. Because of the starving condition of the worms, all substrates of metabolism in both intact and regenerating worms were necessarily derived from the older tissues; loss in weight should be proportional to the amount of the materials metabolized away. Materials which were transferred from the older tissues to the regenerant tissues should not enter into the determination, and thus loss in weight should be proportional to net rather than gross breakdown of substrates. It was found that during the same periods of time regenerating worms lost weight almost twice as rapidly as intact worms. On this second basis, morphogenesis has a metabolic cost nearly the same as the cost of maintenance of the entire worm. The cost estimated from weight loss is thus greater than that estimated from oxygen consumption. Probably not all of the breakdown of substrates is associated with oxygen consumption, and morphogenesis may have an additional cost in terms of activity of anaerobic metabolism or glycolysis.

Analysis of a metabolic cost of morphogenesis may be carried further by comparing the time course of rate of oxygen consumption with the time course of events in regeneration, especially the time course of the processes which were measurable in terms of segments per worm per day. During the period of highest rate of localization, oxygen consumption was only slightly above that of non-regenerating worms. During the earliest stages of differentiation oxygen consumption was almost precisely the same as in non-regenerating worms. This indicates that localization is not particularly costly in terms of aerobic metabolism, while initiation of differentiation appears to cost nothing. The evidence does not indicate that localization and early differentiation are independent of catabolism. Measurements of total oxygen consumption give no indication of the relative activities of different fractions of catabolism, nor do they indicate how much energy may be released through cellular oxidative systems which use some hydrogen acceptor other than molecular oxygen. (Evidence which indicates an "activity metabolism" of localization will be presented in a later paper.)

The peak in rate of oxygen consumption was found to coincide in time with the peak in rate of later differentiation. Furthermore, the earliest time of marked increase in oxygen consumption coincided with the time at which the later stage of differentiation began appearing, first at a low rate, then more rapidly, parallel with increase in rate of oxygen consumption. The marked increase in oxygen consumption in regenerating *T. tubifex* is associated with differentiation, or some process

involved therein, and not with morphogenetic processes in general. It is suggested that differentiation is paid for in terms of metabolically released energy, and that the cost is high. As estimated from increase in oxygen consumption and in loss of weight, this cost must be at least half as great as the cost of maintenance of the entire worm.

Summary

1. Methods of measuring "rate of localization," "rate of early differentiation," and "rate of later differentiation," during posterior regeneration in *Tubifex tubifex* have been described.

2. A method of measuring oxygen consumption of individual worms has been described.

3. Oxygen consumption, as determined according to the above mentioned method and also according to the Warburg manometric method, has been found to proceed at a near normal rate during localization and early stages of differentiation in the first week of regeneration.

4. Markedly increased rate of oxygen consumption has been found associated with maximum rate of later differentiation during the second week of regeneration.

5. No significant cyanide-sensitive fraction of respiration was found at any stage of regeneration.

6. Worms in which regeneration had been inhibited by X-ray treatment showed no increase in oxygen consumption.

7. Loss of weight by the starving regenerating worms was found to be almost twice as great as by the intact worms.

8. The data have been discussed in terms of a metabolic cost of differentiation, which cost would be at least half as great as the metabolic cost of maintenance of the entire worm.

9. It is concluded that the marked increase in aerobic metabolism observed during regeneration in *T. tubifex* is associated with some process or processes involved in differentiation.

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LITERATURE CITED

Barth, L. G., 1938 Quantitative studies of the factors governing the rate of regeneration in Tubularia. *Biol. Bull.*, **74**: 155–177.

Barth, L. G., 1940a. The relation between oxygen consumption and rate of regeneration. *Biol. Bull.*, **78**: 366–374.

Barth, L. G., 1940b. The process of regeneration in hydroids. *Biol. Rev.* **15**: 405–420.

Bodine, Joseph Hall, and John Edgar Boell, 1934. Respiratory mechanism of normally developing and blocked embryonic cells (Orthoptera). *Jour. Cell. Comp. Physiol.*, **5**: 97–113.

Bodine, Joseph Hall, and Loren D. Carlson, 1940. Enzymes in ontogenesis (Orthoptera). X. The effects of temperature on the activity of the naturally occurring and other activators of protyrosinase. *Jour. Cell. Comp. Physiol.*, **16**: 71–84.

Bohr, C., and K. A. Hasselbalch, 1903. Über die Wärmproduktion und den Stoffwechsel des Embryos. *Skand. Archiv. f. Physiol.*, **14**: 398–429.

Brazda, Fred C., and James C. Rice, 1940. The respiratory metabolism of the freshwater oligochaete, Tubifex. *Jour. Cell. Comp. Physiol.*, **16**: 97–102.

Coldwater, Kenneth Bryson, 1930. Action of X-rays on glutathione content and oxygen consumption of normal and regenerating planarians. *Proc. Soc. Exp. Biol. Med.*, **27**: 1031–1033.

Coldwater, Kenneth Bryson, 1933. The effect of sulfhydryl compounds upon regenerative growth. *Jour. Exp. Zoöl.*, **65**: 43–71.

Commoner, Barry, 1940. Cyanide inhibition as a means of elucidating the mechanisms of cellular respiration. *Biol. Rev.*, **15**: 168–201.

Dausend, Kurt, 1931. Über die Atmung der Tubificiden. *Zeitschr. f. vergl. Physiol.*, **14**: 557–608.

Farkas, Koloman, 1903. Beiträge zur Energetik der Ontogenese. III. Über den Energieumsatz des Seidenspinners während der Entwicklung im Ei und während der Metamorphose. *Arch. f. d. ges. Physiol.*, **98**: 490–546.

Fisher, Kenneth C., and Joseph A. Stern, 1942. The separation of an "activity" metabolism from the total respiration of yeast by the effects of ethyl carbamate. *Jour. Cell. Comp. Physiol.*, **19**: 109–122.

Fisher, Kenneth C., R. J. Henry, and E. Low, 1944. The effects of sulfanilamide and azide on oxygen consumption and cell division in the egg of the sea urchin, Arbacia punctulata. *Jour. Pharm. Exp. Therapeutics*, **81**: 58–66.

Harnisch, Otto, 1935. Versuch einer Analyse des Sauerstoffverbrauchs von Tubifex tubifex Müller. *Zeitschr. f. vergl. Physiol.*, **22**: 450–465.

Howland, Ruth B., and Alan Bernstein, 1931. A method for determination of oxygen consumption of a single cell. *Jour. Gen. Physiol.*, **14**: 339–348.

Needham, Joseph, 1931. *Chemical embryology*. Vol. 2: 946–999. The University Press, Cambridge (Eng.).

Stone, Raymond Glenn, 1932. The effects of X-rays on regeneration in Tubifex tubifex. *Jour. Morph.*, **53**: 389–432.

Tangl, Franz, 1909. Embryonale Entwicklung und Metamorphose von energetischen Standpunkte aus betrachtet. *Arch. f. d. ges. Physiol.*, **130**: 55–89.

Tyler, Albert, 1933. On the energetics of differentiation (a comparison of the oxygen consumption of "half" and whole embryos of the sea urchin). *Pubblicazioni della Stazione Zoologica di Napoli*, **13**: 155–161.

Tyler, Albert, 1936. On the energetics of differentiation. III. Comparison of the temperature coefficients for cleavage and later stages in the development of the eggs of some marine animals. *Biol. Bull.*, **71**: 59–81.

Tyler, Albert, 1942. Developmental processes and energetics. *Quart. Rev. Biol.*, **17**: 197–212; 339–353.

Warburg, Otto, 1930. *The metabolism of tumors*. Constable, London.